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# Bioaccessibility of calcium, iron and magnesium in residues of citrus and characterization of macronutrients

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ABSTRACT

The aim of this study was to estimate bioaccessibility of Ca, Fe and Mg in residues of orange, lime, and their mixture, in order to evaluate the effects of cooking in water on mineral bioaccessibility and also to determine the composition of macronutrients and myo-inositol phosphate content. The citrus samples contained on average 9.53 g/100 g moisture, 6.09 g/100 g protein, 3.23 g/100 g ash, 3.15 g/100 g lipids, 34.26 g/100 g insoluble fiber, 27.88 g/100 g soluble fiber and 25.64 g/100 g carbohydrates. The percentage of soluble and dialyzable minerals ranged from 19.36 to 77.33% and from 5.59 to 69.06% for Fe, from 33.34 to 60.84% and 14.71 to − 26.13% for Ca, and from 29.95 to 94.20% and 34.42 to 62.51%, for Mg, respectively. It was verified that cooking influenced the minerals bioaccessibility and increased the dialyzable fraction of Fe and Mg, but decreased the fraction of Ca dialysate, except to orange. No myoinositol phosphate esters were detected. The Principal Component Analysis allowed the separation of different types of citrus residues, but did not discriminate the raw and cooked samples. This study pointed the potential of citrus residue to be used for human consumption and contribute to the necessary dietary minerals and macronutrients, with high content of soluble and insoluble fibers.

## 1. Introduction

Citriculture stands out in the Brazilian economy and according to the Instituto Brasileiro de Geografia e Estatística - IBGE (2014), the annual production of orange and lime are 18 million tons and about 1 million tons, respectively (IBGE, 2014; MAPA, 2015). Citrus fruits contain phenolic compounds, proteins, minerals, vitamins, pigments, volatile compounds (present in the essential oil), lipids, sugars, acids and fibers (Bampidis & Robinson, 2006). This components linked to their nutritional and antioxidant properties make these fruits very important for health (Bermejo, Llosa, & Cano, 2011). However these characteristics can be influenced and affected by edaphoclimatic factors, variety of fruits and the type of fertilization (Barros, Ferreira, & Genovese, 2012). The residues produced from juice extraction correspond to approximately 49% of the weight of the fruit (Mendonça, Conceição, Piedade, Carvalho, & Theodoro, 2006). This residue can be used in the manufacturing of charcoal and in the extraction of soluble fiber (pectin), nevertheless, most of this residue is intended for animal feed and commercialized at low cost (MAPA, 2015; Rezzadori, Benedetti, & 11, 2009), which represents an underutilization of the residue.

The development of technologies and processes with the purpose of adding value to agricultural products and by-products is being studied (Bublitz et al., 2013; Ferreira et al., 2015; Kamiloglu et al., 2017; López-Marcos, Bailina, Viuda-Martos, Pérez-Alvarez, & Fernández-López, 2015). Studies have demonstrated the use of flour and fibers of citrus products as ingredient in biscuits, cereal bars and as fat substitutes in ice cream, contributing to the fiber content and technological properties of these food products (Boff, Crizel, Araujo, Rios, & Flôres, 2013; Ferreira et al., 2015; López-Marcos et al., 2015; Santos et al., 2011).

Minerals are essential to the suitable functioning of the organism, and minerals such as calcium, potassium, iron, zinc, magnesium, manganese, copper, sodium, boron and sulfur are found in the pulp and peel of citrus fruits (Barros et al., 2012; Xu et al., 2008). Micronutrient deficiency still reaches thousands of individuals, in Brazil, such as anemia that still affects about 40% of children (Cozzolino, 2007; Vieira & Ferreira, 2010) and according to the World Health Organization (INACG, WHO,, & UNICEF, 1998) iron deficiency is the main cause of malnutrition in the world; in Brazil calcium and magnesium consumption is still lower than the Dietary Reference Intake

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(DRI) (Cozzolino, 2007).

Digestion simulation tests *in vitro* have long been used to determine nutrient bioaccessibility, and it is an alternative to the methods of digestion simulation *in vivo* (Guerra et al., 2012). Among the different methods for determination of the bioaccessibility of nutrients *in vitro*, the methods of dialysis and solubility are aplied (Akhter, Saeed, Irfan, & Malik, 2012; Xia, Wang, Xu, Mei, & Li, 2017). The minerals tend to suffer interactions that determine changes in their absorption, interfering in the nutritional quality of foods. The availability of some minerals decreases in the presence of antinutritional factors, the main cause of this reduction is phytate (Schons, 2009), and oxalates, tannins and fibers are also included as possible interferents in the availability of minerals (Amalraj & Pius, 2015; Bosscher, Caillie-Bertrand, Cauwenbergh, & Deelstra, 2003). Phytic acid can complex itself with minerals forming insoluble compounds, decreasing the bioavailability and absorption of these minerals by the organism (Kies, Jonge, Kemme, & Jongbloed, 2006; Pereira, 2010). Some methods of heat treatment have been used to reduce phytate content in foods (Embaby, 2011; Karkle & Beleia, 2010).

Studies with the objective of estimating the bioaccessibility of minerals in foods (Fe, Zn, Ca, Cu, P, Mg, Mn) through solubility and dialysis methods have already been published (Argyri et al., 2011; Cámara, Amaro, Barberá, & Clemente, 2005; Xia, Tao, et al., 2017). Notwithstanding, the bioaccessibility of important minerals in the residues of citrus products, such as iron, calcium and magnesium was not reported. These citrus residues can be used in food, providing nutrients and contributing in functional properties of food, however they may contain antinutritional factors such as phytates.

The aim of this study was to determine the centesimal composition of citrus residues (lime, orange and mixture of limes and oranges), to study the bioaccessibility of minerals (solubility and dialysis methods) in citrus residue originated from the juice industry, and to verify the behavior of calcium, iron and magnesium in simulated gastrointestinal digestion, in order to estimate the behavior of these minerals in the human digestive system. The fractions of *myo*-inositol phosphate (phytates) and the effects of heat treatment on the release of minerals were also evaluated. All data collected may contribute to the valorization of this by-product of the agribusiness and point to the possibility of its use as an ingredient in formulations of foods containing bioaccessible minerals.

## 2. Material and methods

### 2.1. Equipments

The samples were milled in a grinder (model A11 – Ika, Germany) and weighed in an analytical balance (model AP210-0 - Ohaus). The mineralization of samples was performed using a block digester (M242 model – Quimis, Brazil) and they were subsequently dissolved in ultrasound bath (model 1510-Branson). For spectrophotometric determinations, a flame atomic absorption spectrometer (FAAS), AAnalyst 200 model, with a deuterium lamp for correction of the background radiation and hollow cathode lamps for determination of iron, calcium and magnesium (Perkin Elmer) was used.

The centesimal composition was determined using an drying oven with forced air circulation (model 400-3ND - Nova Ética, Brazil), a Wagner type test tube shaker (model MA 160/50/*CF* — Marconi, Brazil), a muffle furnace (model 6318M24, Quimis, Brazil), a block digestor (TE4025 model, Tecnal, Basil), a nitrogen distiller (Tecnal, model TE 036-1, Brazil), metabolic bath (Dubnoff, model MA093, Marcone, Brazil) and a vacuum pump (model 089 CAL, Merse, Brazil).

For the analyses of bioaccessibility, a refrigerated centrifuge (model CR21GII, Hitachi), a pH meter (Digimed DM 22), a metabolic bath (Dubnoff, MA 093, Marcone, Brazil) and a lyophilizer (LS, Terroni, Brazil) were used.

### 2.2. Reagents

For mineralization, nitric acid (≥ 65%, Sigma-Aldrich) and hydrogen peroxide (29%, Synth) of analytical purity grade were used. For mineral analysis, standard solutions of iron, calcium and magnesium (Sigma-Aldrich), 1000 mg/L, quantitative filter paper (Nalgon, 9 cm diameter) ashes free, and qualitative filter papers (Nalgon) were used. In the bioaccessibility assays were used enzymes from Sigma Chemical Co. (St. Louis, USA): bile, from bovine and ovine (B8381, EC 232-369-0), pepsin, from porcine gastric mucosa (P7000, EC 3.4.23.1), and pancreatin, from porcine pancreas (P7545, EC 232-468-9). For centesimal composition assays, a Total Dietary Fiber assay kit (TDF-100A, Missouri, USA) from Sigma Chemical Co., sodium bicarbonate (97.3%, Synth), hydrochloric acid (28%, Chemco), chloroform (99,8%, Synth), methanol (99.8%, Synth), sodium sulphate (99%, Merk), potassium sulphate (99%, Synth), copper sulphate (98%, Synth), sulfuric acid (98%, Synth), boric acid (99.5%, Synth), methyl red (PA, Synth), bromocresol green (PA, Synth), sodium hydroxide (98%, Synth), dibasic sodium phosphate (98%, Synth), monobasic sodium phosphate (99%, Synth), ethanol (99.5%, Synth), acetone (99.5%, Chemis), acidic celite (acid washed Celite, Sigma- Aldrich), formic acid (98%, Merk) and phytic acid sodium salt hidrate (Sigma-Aldrich) were used.

### 2.3. Samples

Dry residues of Pera orange, Tahiti lime and a mixture (Pera orange, Hamlin orange and Tahiti lime, (1:1:1 w/w/w)) donated by local industry (SP, Brazil) were used. The provided residue consisted of citrus peels (albedo and flavedo) and seeds, the essential oil had already been removed. Approximately 100 g of each sample was milled and homogenized for the analyses, resulting in a flour.

### 2.4. Analysis of the centesimal composition

The centesimal composition for the citrus residue was determined by evaluating the levels of moisture, ash, fibers (total, soluble and insoluble, 991.43 method), proteins ($N = 6.25$) in accordance with AOAC (2006) and Prosky et al. (1985), and carbohydrates by difference [100 – (moisture – lipid – ash – protein - total fibers)]. The lipids were determined according to Bligh and Dyer (1959). The results were expressed on wet basis, and the assays were performed in triplicate, except to fibers ($n = 4$).

### 2.5. Heat treatment

In order to evaluate the heat treatment effect (cooking) on the bioaccessibility of minerals in the samples of Pera orange, Tahiti lime and mixture, samples were cooked in ultrapure water boiling at 1:20 w/v ratio (sample:water) for 20 min. Then the cooked samples were cooled, frozen at − 40 °C, lyophilized (sample and cooking water) for 24 h up to a pressure of < 200 μHg, and subjected to an analysis of solubility and dialysis.

### 2.6. Bioaccessibility of minerals

The bioaccessibility of minerals was evaluated with the use of methods of solubility and dialysis. Initially, the total amount of the minerals calcium (Ca), iron (Fe) and magnesium (Mg) was determined in the samples. The samples were mineralized, analyzed and quantified in Spectrometer Atomic Absorption (FAAS) according to Silva, Orlando, Rebellato, and Pallone (2016).

#### 2.6.1. Solubility and dialysis

The methodology for the solubility and dialysis assays was performed according to the method described by Jovaní, Barberá, Farré, and Aguilera (2001), with modifications. Approximately 1 g of each